

CLAIMS

What is claimed is:

1. A method for the expression of a coding region of interest in a *Bacillus sp* comprising:
 - 5 a) providing a transformed *Bacillus sp* cell having a chimeric gene comprising a nucleic acid fragment comprising the promoter region of a *Bacillus* gene operably linked to a coding region of interest expressible in a *Bacillus sp*, wherein the nucleic acid fragment comprising the promoter region of a *Bacillus* gene is selected from the group consisting of *narGHJI*, *csn*, *yncM*, *yvyD*, *yvaWXY*, *ydjL*, *sunA*, and *yolIJK* and homologues thereof; and
 - 10 b) growing the transformed *Bacillus sp* cell of step (a) in the absence of oxygen wherein the chimeric gene of step (a) is expressed.
- 15 2. A method for the expression of a coding region of interest in a *Bacillus sp* comprising:
 - a) providing a transformed *Bacillus sp* cell having a chimeric gene comprising a nucleic acid fragment comprising the promoter region of a *Bacillus* gene operably linked to a coding region of interest expressible in a *Bacillus sp*, wherein the nucleic acid fragment comprising the promoter region of a *Bacillus* gene is selected from the group consisting of *narGHJI*, *csn*, *yncM*, *yvyD*, *yvaWXY*, *ydjL*, *sunA*, and *yolIJK* and homologues thereof;
 - 20 b) growing the transformed *Bacillus sp* cell of step (a) in the presence of oxygen whereby the cell density is increased; and
 - 25 c) removing oxygen from the transformed *Bacillus sp* cell of step (b) whereby the chimeric gene is expressed.
- 30 3. A method according to Claim 2 wherein after step (c) oxygen is re-supplied to the transformed *Bacillus sp* cell.
4. A method according to either of Claims 1 or 2 wherein the nucleic acid fragment comprising the promoter region of a *Bacillus* gene is selected from the group consisting of SEQ ID NOs:1-15.
- 35 5. A method for the expression of a coding region of interest in a *Bacillus sp* comprising:
 - a) providing a transformed *Bacillus sp* cell having a chimeric gene comprising a nucleic acid fragment comprising the promoter region of a *Bacillus* gene operably linked to a coding region of

interest expressible in a *Bacillus sp*, wherein the nucleic acid fragment comprising the promoter region of a *Bacillus* gene is selected from the group consisting of *feuABC*, *ykuNOP*, and *dhbABC*, and homologues thereof; and

5 b) growing the transformed *Bacillus sp* cell of step (a) in the absence of oxygen and in the presence of nitrite wherein the chimeric gene of step (a) is expressed.

6. A method according to Claim 5 wherein the nucleic acid fragment comprising the promoter region of a *Bacillus* gene is selected from the group consisting of SEQ ID NOs:16-24.

10 7. A method according to Claim 6 wherein the concentration of nitrite is from about 1mM to about 10 mM.

8. A method for the expression of a coding region of interest in a *Bacillus sp* comprising:

15 a) providing a transformed *Bacillus sp* cell having a chimeric gene comprising a nucleic acid fragment comprising the promoter region of a *Bacillus* gene operably linked to a coding region of interest expressible in a *Bacillus sp*, wherein the nucleic acid fragment comprising the promoter region of a *Bacillus* gene is selected from the group consisting of *ycgMN*, *dhaS rapF*, *rapG*, *rapH*, *rapK*, *yqhIJ*, *yveKLMNOPQST*, *yhfRSTUV*, *csn*, *yncM*, *yvyD*, *yvaWXY*, *ydjL*, *sunA*, and *yolIJK*, and homologues thereof; and

20 b) growing the transformed *Bacillus sp* cell of step (a) in the presence of oxygen until the cell reaches about T0 of the stationary phase wherein the chimeric gene of step (a) is expressed.

25 9. A method according to Claim 8 wherein the nucleic acid fragment comprising the promoter region of a *Bacillus* gene is selected from the group consisting of SEQ ID NOs:75, 76, 25-49, and 5-15.

30 10. A method for the expression of a coding region of interest in a *Bacillus sp* comprising:

35 a) providing a transformed *Bacillus sp* cell having a chimeric gene comprising a nucleic acid fragment comprising the promoter region of a *Bacillus* gene operably linked to a coding region of interest expressible in a *Bacillus sp*, wherein the nucleic acid fragment comprising the promoter region of a *Bacillus* gene is

selected from the group consisting of *acoABCL*, and *glvAC*, and homologues thereof; and

5 b) growing the transformed *Bacillus sp* cell of step (a) in the presence of oxygen until the cell reaches about T1 of the stationary phase wherein the chimeric gene of step (a) is expressed.

11. A method according to Claim 10 wherein the nucleic acid fragment comprising the promoter region of a *Bacillus* gene is selected from the group consisting of SEQ ID NOs:41-44 and 50-51.

10 12. A method for the expression of a coding region of interest in a *Bacillus sp* comprising:

15 a) providing a transformed *Bacillus sp* cell having a chimeric gene comprising a nucleic acid fragment comprising the promoter region of a *Bacillus* gene operably linked to a coding region of interest expressible in a *Bacillus sp*, wherein the nucleic acid fragment comprising the promoter region of a *Bacillus* gene is selected from the group consisting of *yxjCDEF*, *yngEFGHI*, *yjmCDEFG*, *ykfABCD*, and *yodOPRST*; and homologues thereof; and

20 b) growing the transformed *Bacillus sp* cell of step (a) in the presence of oxygen until the cell reaches about T3 of the stationary phase wherein the chimeric gene of step (a) is expressed.

13. A method according to Claim 12 the nucleic acid fragment comprising the promoter region of a *Bacillus* gene is selected from the group consisting of SEQ ID NOs:52-74.

25 14. A method according to any of Claims 1, 2 or 3 wherein the expression of the chimeric gene is down-regulated at T0 of the stationary phase.

15. A method according to any one of Claims 1, 2, 3, 4, 8, 10 and 12

30 wherein the *Bacillus sp.* cell is selected from the species consisting of *Bacillus subtilis*, *Bacillus thuringiensis*, *Bacillus anthracis*, *Bacillus cereus*, *Bacillus brevis*, *Bacillus megaterium*, *Bacillus intermedius*, *Bacillus thermoamyloliquefaciens*, *Bacillus amyloliquefaciens*, *Bacillus circulans*, *Bacillus licheniformis*, *Bacillus macerans*, *Bacillus sphaericus*, *Bacillus stearothermophilus*, *Bacillus laterosporus*, *Bacillus acidocaldarius*, *Bacillus pumilus*, and *Bacillus pseudofirmus*.

35 16. The method according to any one of Claims 1, 2, 3, 4, 8, 10 and 12, wherein the coding region of interest is selected from the group consisting of *crtE*

crtB, pds, crtD, crtL, crtZ, crtX crtO, phaC, phaE, efe, pdc, adh, genes encoding limonene synthase, pinene synthase, bornyl synthase, phellandrene synthase, cineole synthase, sabinene synthase, and taxadiene synthase.

17. A method for monitoring the state of the cell metabolism of a *Bacillus* sp. culture comprising:

- a) providing a culture of actively growing *Bacillus* sp. cells; and
- b) measuring the expression levels of a pool of genes isolated from the *Bacillus* cells of step (a), the pool of genes comprising *narGHJI, feuABC, ykuNOP, dhbABC, ydjL, sunA, yolIJK, csn, yncM, yyvD, yvaWXY, yhfRSTUV, yveKLMNOPQST, dhaS, rapF, rapG, rapH, rapK, ycgMN, yqhIJ, glvAC, acoABCL, yxjCDEF, yngEFGHI yjmCDEFG, ykfABCD, yodOPRST, alsT, and yxeKLMN*, and homologues thereof.

18. A method according to Claim 17 wherein a pool of genes isolated from the *Bacillus* cells is selected from the group consisting of SEQ ID NOs:1-81.

19. A method according to Claim 17 wherein the measuring of gene expression levels is accomplished using a format selected from the group consisting of northern blots, nuclease protection assay or primer extension assays.

20. A method according to Claim 19 wherein the measuring of gene expression levels is accomplished using a nucleic acid microarray having the genes *narGHJI, feuABC, ykuNOP, dhbABC, ydjL, sunA, yolIJK, csn, yncM, yyvD, yvaWXY, yhfRSTUV, yveKLMNOPQST, dhaS, rapF, rapG, rapH, rapK, yqhIJ, glvAC, acoABCL, yxjCDEF, yngEFGHI yjmCDEFG, ykfABCD, yodOPRST, alsT, and yxeKLMN*, and homologues thereof, contained therein.

21. A method according to Claim 17 wherein the *Bacillus* sp. cell is selected from the species consisting of *Bacillus subtilis, Bacillus thuringiensis, Bacillus anthracis, Bacillus cereus, Bacillus brevis, Bacillus megaterium, Bacillus intermedius, Bacillus thermoamylolyticus, Bacillus amylolyticus, Bacillus circulans, Bacillus licheniformis, Bacillus macerans, Bacillus sphaericus, Bacillus stearothermophilus, Bacillus laterosporus, Bacillus acidocaldarius, Bacillus pumilus, and Bacillus pseudofirmus*.

22. A method according to Claim 17 wherein the actively growing culture is grown in the absence of oxygen and the expression of genes *narGHJI, ydjL, sunA, yolIJK, csn, yncM, yyvD, and yvaWXY* are up-regulated in the log phase.

23. A method according to Claim 17 wherein the actively growing culture is grown in the absence of oxygen and in the presence of nitrite and the expression of genes *feuABC, ykuNOP, and dhbABC* are up-regulated in the log phase.

24. A method according to either of Claims 22 or 23 wherein the expression of genes *narGHJI* is down-regulated at about T0 of the stationary phase.

25. A method according to Claim 17 wherein the actively growing culture
5 is grown in the presence of oxygen and the expression of genes *ycgMN*, *yqhIJ*,
ydjL, *sunA*, *yolIJK*, *csn*, *yncM*, *yvyD*, *yvaWXY*, *yhfRSTUV*, *yveKLMNOPQST*,
dhaS, *rapF*, *rapG*, *rapH*, *rapK*, are up-regulated at about T0 of the stationary phase.

26. A method according to Claim 17 wherein the actively growing culture
10 is grown in the presence of oxygen and the expression of genes, *acoABCL* and
glvAC are up-regulated at about T1 of the stationary phase.

27. A method according to Claim 17 wherein the actively growing culture
is grown in the presence of oxygen and the expression of genes, *yxjCDEF*,
yngEFGHI *yjmCDEFG*, *ykfABCD*, and *yodOPRST* are up-regulated at about T3 of
15 the stationary phase.

28. A method according to Claim 17 wherein the actively growing culture
is grown in the presence of oxygen and the expression of genes, *alsT* and
yxeKLMN are down-regulated at stationary phase or under nutrient-limiting
conditions.